Electronic Supplementary Information

Novel template-assembled oligosaccharide clusters as epitope mimics for HIV-neutralizing antibody 2G12. Design, synthesis, and antibody binding study

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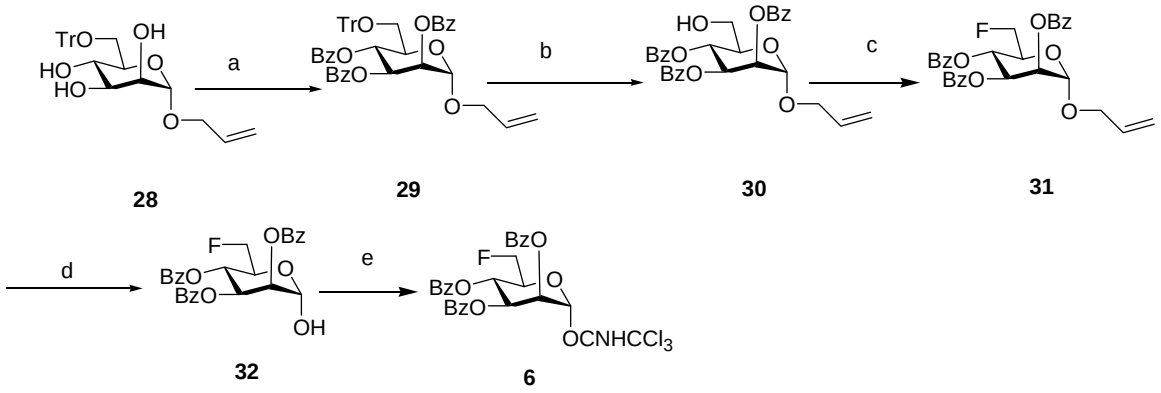


Reagents and conditions: *a) BzCl/Py, 95%; b) 80% AcOH, 60 °C, 93%, c) DAST/CH_2Cl_2, 53%; d) $PdCl_2$/MeOH, 89%; e) CCl_3CN,DBU, 80%.*

Allyl 6-O-triphenylmethyl-2,3,4,-tri-O-benzoyl-α-D-mannopyranoside (29)

To a solution of **28** (300 mg, 0.65 mmol) in dry pyridine (5 mL) was added dropwise a solution of BzCl (1 mL) in pyridine (2 mL) at 0 ºC. The mixture was then stirred at rt overnight. The reaction was quenched by adding ice-water, extracted with CH_2Cl_2. The organic layer was washed with 1N HCl, brine and water, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (Hexanes/EtOAc, 10:1) to give compound **29** (477 mg, 95%) as a white solid. δ_H (500 MHz, $CDCl_3$): 8.21-7.12 (m, 30 H, Ph), 6.10 (t, 1 H, J = 9.0 Hz, H-4), 6.10-6.02 (m, 1 H, C*H*=CH_2), 5.88 (dd, 1 H, J = 3.5, 9.0 Hz, H-3), 5.78 (dd, 1 H, J = 1.5, 3.2 Hz, H-2), 5.45 (dd, 1 H, J = 1.5, 16.0 Hz, ½ CH_2=), 5.35 (dd, 1 H, J = 1.5, 10.0 Hz, ½ CH_2=), 5.26 (d, 1 H, J = 1.5 Hz, H-1), 4.46-4.20(m, 3 H, H-5, OCH_2CH=CH_2), 3.5-3.4 (m, 2 H, H-6). δ_C ($CDCl_3$, 125 MHz): 165.7, 165.6, 165.2, 143.8, 133.8, 133.5, 133.4, 133.1, 133.0, 133.0, 129.8, 129.7, 128.7, 128.6, 128.5, 128.3, 128.2, 128.7, 126.9, 118.2, 96.7, 70.9, 70.6, 70.5, 68.6, 67.1, 62.3.

Allyl 2,3,4,-tri-O-benzoyl-α-D-mannopyranoside (30)

A solution of **29** (560 mg, 0.73 mmol) in an aqueous AcOH (80%, 20 mL) was stirred at 70 ºC for 3 h. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography (Hexanes/EtOAc 4:1) to give compound **30** (361 mg, 93%) as a white solid. δ_H (500 MHz, $CDCl_3$): 8.17-7.29 (m, 15 H, Ph-H), 6.08 (dd, 1 H, J = 3.0, 9.0 Hz, H-3), 6.03 (m, 1 H, C*H*=CH_2), 5.92 (t, 1 H, J = 9.5 Hz, H-4), 5.77 (dd, 1 H, J = 1.5, 3.0 Hz, H-2), 5.44 (dd, 1 H, J = 1.5, 17 Hz, ½ CH_2=), 5.30 (dd, 1 H, J = 1.5, 11.0 Hz, ½ CH_2=), 5.22 (br. s, 1 H, H-1), 4.37 (m, 1 H, H-5), 4.19 (m, 2 H, C*H*$_2$CH=CH_2), 3.87 (m, 2 H, H-6). δ_C ($CDCl_3$, 125 MHz): 166.6, 165.6, 165.5, 133.7, 133.6, 133.2, 133.1, 130.0, 129.9, 129.7, 129.3, 129.2, 128.8, 128.7, 128.6, 128.5, 128.4, 118.4, 96.9, 71.1, 70.7, 69.8, 68.9, 67.4, 61.4.

Allyl 2,3,4,-tri-O-benzoyl-6-deoxy-6-fluoro-α-D-mannopyranoside (31)

To a stirred solution of **30** (155 mg, 0.29 mmol) in dry CH_2Cl_2 (5 mL) at -40 ºC was added DAST (94 mg, 0.076 mL, 0.58 mmol) over 30 min. The reaction mixture was stirred at this temperature for another 30 min then at room temperature for 1h, when TLC indicated the completion of fluorination. After cooling to -20 ºC, MeOH and aqueous $NaHCO_3$ were added to quench the reaction. The mixture was filtered and the filtrate was extracted with CH_2Cl_2. The organic layer was washed with brine and water, dried, and concentrated. The residue was purified by silica gel column chromatography (Hexanes/Hexanes 5:1) to give compound **31** (82 mg, 53%) as a white solid. δ_H (500 MHz, $CDCl_3$): 8.16-7.30 (m, 15 H, Ph), 6.02 (m, 1 H, C*H*=CH_2), 5.96 (m, 2 H, H-3, H-4), 5.76 (dd, 1 H, J = 1.3, 3.0 Hz, H-2), 5.45 (dd, 1 H, J = 1.5, 17 Hz, ½ CH_2=), 5.36 (dd, 1 H, J = 1.3, 11 Hz, ½ CH_2=), 5.21 (d, 1 H, J = 1.3 Hz, H-1), 4.8-4.6 (m, 2 H, H-6), 4.4-4.2 (m. 2 H, C*H*$_2$CH=CH), 4.35 (m, 1 H, H-5); δ_C ($CDCl_3$, 125 MHz): 165.6, 165.5, 165.4, 133.6, 133.2, 133.0, 130.0, 129.9, 129.8, 129.3, 129.1, 128.9, 128.7, 128.5, 128.3, 128.1, 118.6, 96.7, 81.7 (d, J_{C-F} = 175 Hz, C-6), 70.5, 69.9, 69.8, 69.7, 68.9, 66.4, 66.3.

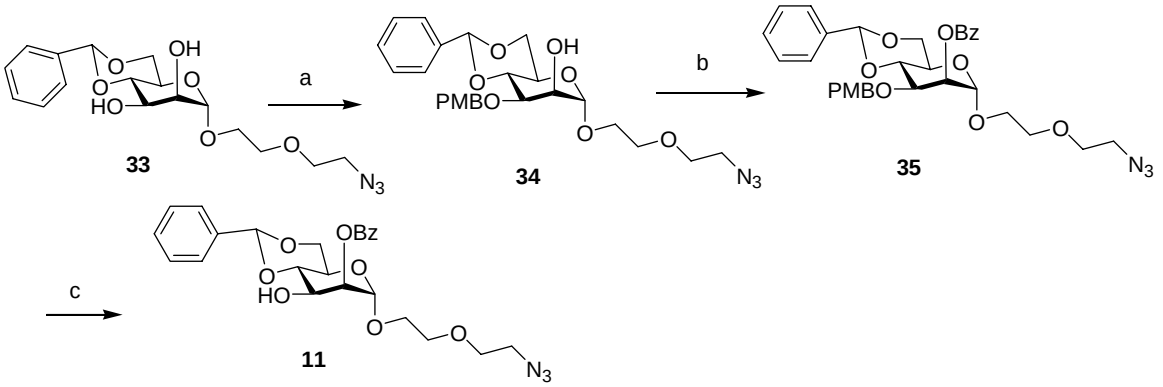
2,3,4,-Tri-O-benzoyl-6-deoxy-6-Fluoro-α-D-mannopyranose (32)

To a solution of **31** (450 mg, 0.84 mmol) in anhydrous MeOH (30 mL) was added $PdCl_2$ (80 mg), the mixture was stirred at 40 °C. After 4 h, TLC (Hexanes/EtOAc 2:1) indicated the complete removal of the allyl group. The mixture was filtered through a pad of Celite, and the filtrate was concentrated. The residue was purified by flash silica gel column chromatography (Hexanes/EtOAc 4:1) to give compound **32** (370 mg, 89%) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.18-7.30 (m, 15 H, Ph), 6.10 (dd, 1 H, J = 3.5, 10.0 Hz, H-3), 6.02 (t, 1 H, J = 9.8 Hz, H-4), 5.83 (dd, 1 H, J = 1.4, 3.4 Hz, H-2), 5.63 (dd, 1 H, J = 1.8, 5.0 Hz, H-1), 4.79-4.58 (m, 3 H, H-5, H-6), 4.44 (d, 1 H, J = 5.0 Hz, OH); δ_C ($CDCl_3$, 125 MHz): 165.8, 165.7, 165.7, 133.7, 133.6, 133.4, 133.3, 130.0, 130.0, 129.9, 129.8, 129.8, 129.7, 129.3, 129.0, 128.9, 128.8, 128.6, 128.5, 128.3, 92.4, 81.8 (d, J_{C-F} = 175 Hz, C-6), 71.1, 71.0, 69.9, 69.8, 69.7, 69.6, 69.6, 69.5, 66.4.

2, 3,4-tri-O-Benzoyl-6-deoxy-6-fluoro-α-D-mannopyranosyl trichloroacetimidate (6)

To a solution of **32** (250 mg, 0.506 mmol) in CH_2Cl_2 (10 mL) were added trichloroacetonitrile (0.5 mL) and DBU (0.1 mL). The mixture was stirred at rt overnight and then concentrated under vacuum. The residue was purified by flash silica gel column chromatography (Hexanes/EtOAc 4:1) to give compound **6** (259 mg, 80 %) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.95 (s, 1 H, NH=$CCCl_3$), 8.18-7.30 (m, 15 H, Ph), 6.65 (d, 1 H, J = 1.8Hz, H-1), 6.12 (t, 1 H, J = 10.0Hz, H-4), 6.03 (dd, 1 H, J = 3.5, 10.0 Hz, H-3), 5.98 (dd, 1 H, J = 1.5, 3.5 Hz, H-2), 4.78-4.64 (m, 2 H, H-6), 4.65-4.48 (ddt, 1 H, J = 3.5, 10, 22.5 Hz, H-5); δ_C ($CDCl_3$, 125 MHz): 165.5, 165.4, 165.3, 159.8, 133.8, 133.7, 133.4, 130.1, 129.9, 129.8,128.9, 128.8, 128.7, 128.7, 128.6, 128.4, 94.7, 90.6, 81.1 (d, J_{C-F} = 175 Hz, C-6), 72.4, 72.3, 69.7, 68.7, 65.5, 65.4.

Synthesis of 2-(2-Azidoethoxy)ethyl 4,6-O-benzylidene-2-O-benzoyl -α-D-mannopyranoside (11)



***Reagents and conditions**: a) 1: Bu_2SnO, MeOH, reflux, 2) PMBCl, TBAI, CsF, Toluene, reflux, 87%; b) BzCl/Py, 90%; c) DDQ, CH_2Cl_2/H_2O, 50%*

2-(2-Azidoethoxy)ethyl-4,6-O-benzylidene-3-O-p-methoxybenzyl-α-D-mannopyranoside (34)

A mixture of **33** (200 mg, 0.525 mmol) and Bu_2SnO (170 mg, 0.682 mmol) in MeOH (10 mL) was heated to reflux overnight, then the solvent was removed under reduced pressure. The residue was dissolved in anhydrous toluene and PMBCl (246 mg, 1.57 mmol), TBAI (38 mg, 0.102 mmol) and CsF (151 mg, 1 mmol) were added. The mixture was heated under reflux for 4 h when TLC indicated the disappearance of the starting material. The reaction mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$, brine and water, dried over Na_2SO_4, and filtered. The filtrate was concentrated and the residue was purified by flash silica gel column chromatography (Hexanes/EtOAc 4:1) to give compound **34** (230 mg, 87 %) as a yellow syrup. δ_H (500 MHz, $CDCl_3$): 7.6-6.9 (m, 9 H, Ph), 5.7 (s, 1 H, PhC*H*=), 4.95 (d, 1 H, J = 1.0 Hz, H-1), 4.83 (d, 1 H, J = 11.5 Hz, ½ CH_2Ph), 4.69 (d, 1 H, J = 11.5 Hz, ½ CH_2Ph), 4.31 (dd, 1 H, J = 3.0, 9.0 Hz, H-6), 4.15 (m, 2 H, H-2, H-4), 3.98 (dd, 1 H, J = 3.0, 9.5 Hz, H-3), 3.94- 3.82 (m, 6 H), 3.73 -3.64 (m, 5 H), 3.42 (t, 2 H, J = 5.0 Hz, CH_2N_3); δ_C ($CDCl_3$, 125 MHz): 159.4, 137.7, 130.2, 129.6, 129.0, 128.3, 126.1, 113.9, 101.6, 100.2, 78.9, 75.3, 72.8, 70.2, 70.1, 70.0, 68.9, 66.8, 63.4, 55.3, 50.7.

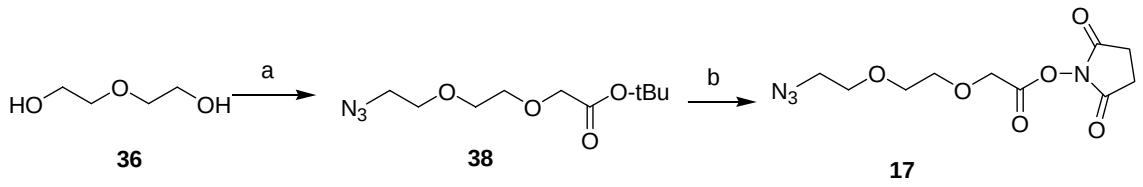
2-(2-Azidoethoxy)ethyl-4,6-O-benzylidene-2-O-benzoyl-3-O-p-methoxybenzyl-α-D-mannopyranoside (35)

To a solution of **34** (200 mg, 0.412 mmol) in pyridine (2 mL) was added a solution of benzoyl choloride (0.2 mL) in pyridine (0.2 mL). The mixture was stirred at 0 ºC for 4 h, and ice water was added to quench the reaction. After dilution with CH_2Cl_2, the mixture was washed with brine and water. The organic layer was dried over Na_2SO_4, filtered, and the filtrate was concentrated. The residue was purified by flash silica gel column chromatography (Hexanes/EtOAc 4:1) to give compound **35** (230 mg, 90%) as a white foam. δ_H (500 MHz, $CDCl_3$): 8.2-6.8 (m, 14H, Ph), 5.73 (s, 1 H, PhC*H*=), 5.68 (dd, 1 H, J= 3.0, 1.5 Hz, H-2), 5.04 (d, 1 H, J = 1.5 Hz, H-1), 4.73 (d, 1 H, J = 12 Hz, ½ $PhCH_2$), 4.67 (d, 1 H, J = 12 Hz, ½ $PhCH_2$), 4.35 (dd, 1 H, J = 5.0, 10.0 Hz, H-6), 4.22 (t, 1 H, J = 9.0 Hz, H-4), 4.20 (dd, 1 H, J = 3.0, 9.0 Hz, H-3), 4.03 (dt, 1 H, J = 4.5, 10.5 Hz, H-5), 3.98- 3.70 (m, 7 H), 3.80 (s, 3 H, CH_3O), 3.45 (t, 2 H, J = 5.0 Hz, CH_2N_3). δ_C ($CDCl_3$, 125 MHz): 165.8, 159.2, 137.6, 133.3, 130.2, 130.2, 130.0, 129.8, 129.3, 129.0, 128.5, 128.4, 128.2, 126.2, 113.7, 101.7, 99.0, 78.7, 73.7, 71.7, 70.3, 70.2, 70.1, 68.9, 67.1, 64.0, 55.4, 50.7.

2-(2-Azidoethoxy)ethyl-4,6-O-benzylidene-2-O-benzoyl-α-D-mannopyranoside (11)

To a solution of **35** (200 mg, 0.33 mmol) in CH_2Cl_2/H_2O (5 mL, 9/1) was added DDQ (90 mg), and the mixture was stirred at rt for 2 h. The mixture was then concentrated under vacuum and the residue was subject to column chromatography to give compound **11** (80 mg, 50%) as white solid. δ_H (500 MHz, $CDCl_3$): 8.2-7.4 (m, 10 H, Ph), 5.71 (s, 1 H, PhC*H*=), 5.68 (dd, 1 H, J = 1.5, 3.8 Hz, H-2), 5.06 (d, 1 H, J = 1.5 Hz, H-1), 4.45 (dd, 1 H, J = 3.5, 9.3 Hz, H-3), 4.37 (dd, 1 H, J = 4.5, 11.0 Hz, H-6), 4.10 (t, 1 H, J = 9.0 Hz, H-4), 4.04 (m, 1 H, H-5), 3.96-3.72 (m, 7 H), 3.47 (t, 2 H, J = 4.5Hz, CH_2N_3). δ_C ($CDCl_3$, 125 MHz): 166.1, 137.2, 133.5, 130.0, 129.6, 129.3, 128.5, 128.4, 126.3, 102.2, 98.8, 79.5, 72.6, 70.3, 70.2, 68.9, 67.6, 67.3, 63.5, 50.8. ESI-MS: m/z: calcd for $C_{24}H_{27}N_3O_8$: 485.5; found: 486.5 $[M+H]^+$, 508.2 $[M+Na]^+$.

Synthesis of succinimidyl 8-azido-3,6-dioxaoctanoate (17)



Reagents and conditions: *a) 1) $BrCH_2COOt\text{-}Bu$, NaH/THF 2) TsCl, DIPEA, CH_2Cl_2; 3) NaN_3, DMF, 83%; b) 1)TFA/CH_2Cl_2 1:1, 90% , 2) NHS, EDCI, CH_2Cl_2*

tert-Butyl 8-azido-3,6-dioxaoctanoate (38)

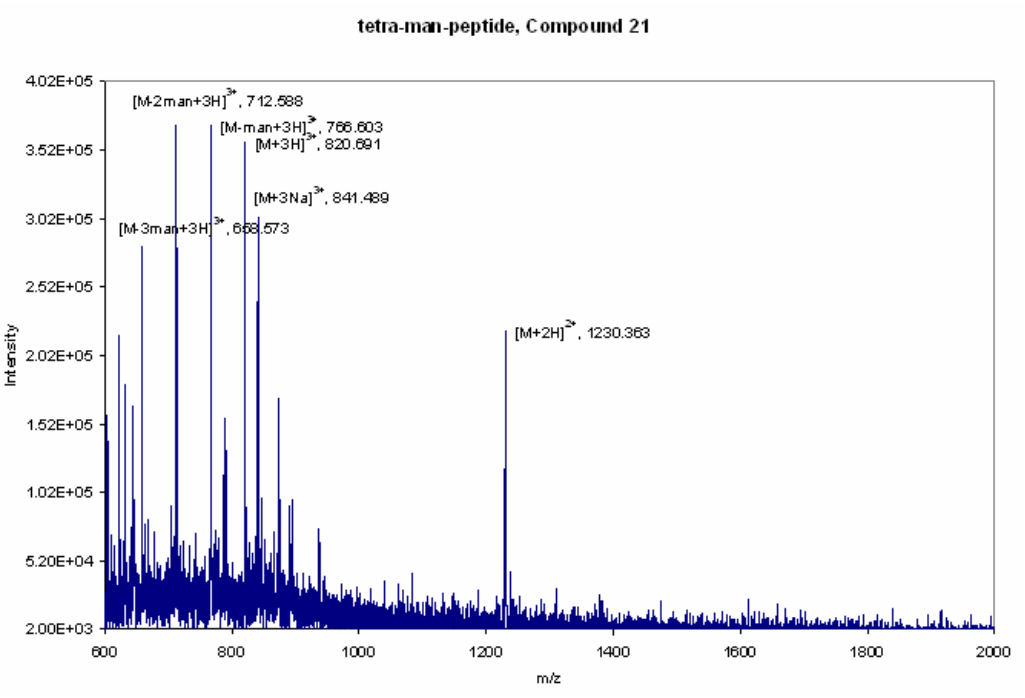
Sodium hydride (60% suspension in mineral oil, 2.72 g, 68.0 mmol) was added in small portions to a solution of diethylene glycol (**36**) (23.0 g, 0.22 mol) in 100 mL of anhydrous THF at 0 °C. After stirring for 30 min, tert-butyl bromoacetate (13.26 g, 68.0 mmol) was added dropwise to the reaction mixture within 10 min at 0 °C, and the mixture was stirred at rt for 2 h. The volatiles were removed in vacuo. The oily residue was partitioned between brine and CH_2Cl_2. The organic layer were separated, dried over $MgSO_4$, and filtered. The filtrate was concentrated to give 38, which was used in the next step without further purification.

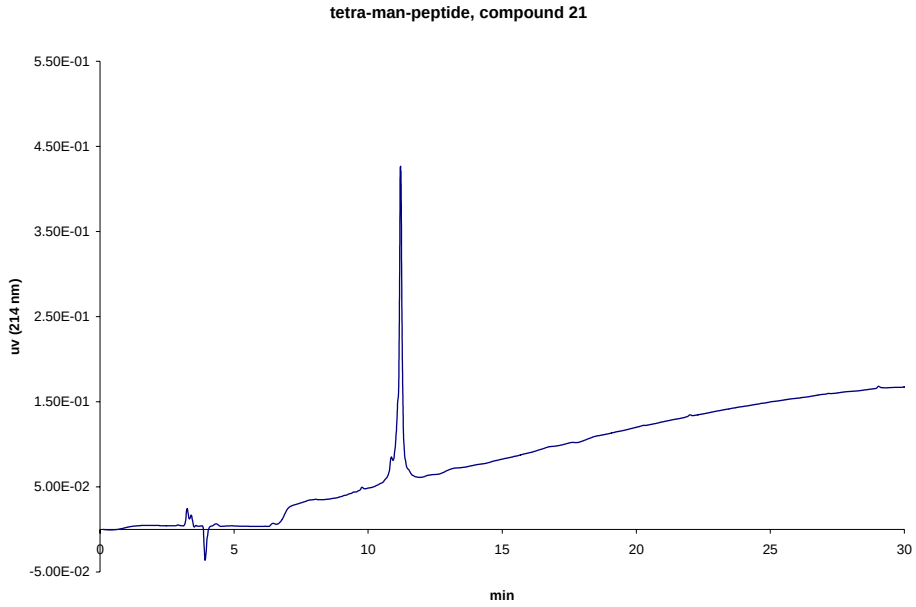
To a solution of the above compound in CH_2Cl_2 (100 mL) were added TsCl (40.0g, 0.21mol) and DIPEA (27g, 0.21mol). The mixture was stirred at rt. After 4 h, the reaction was quenched by 2N NaOH. The organic layer was separated and washed with brine and water, dried over $MgSO_4$, and filtered. The filtrate was concentrated and the residue was subject to column chromatography to give the tosylated compound (1.8 g). The compound was dissolved in dry DMF (100 mL) and NaN_3 (0.62 g, 9.5 mmol) was added. The mixture was stirred at 40 ºC for 24 h and then concentrated under vacuum. The residue was dissolved in EtOAc and washed with water. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated and the residue was subject to column chromatography to give compound **38** (0.72 g, 83%) as a colorless syrup. δ_H (500 MHz, $CDCl_3$): 4.09 (s, 2 H, C*H*$_2$COOtBu), 3.81-3.74 (m, 6 H, $CH_2OCH_2CH_2O$), 3.46 (t, 2 H, J = 5.0 Hz, CH_2N_3), 1.54 (s, 9 H, Ot-Bu); δ_C ($CDCl_3$, 125 MHz): 169.6, 81.6, 70.8, 70.7, 70.0, 69.1, 50.7, 28.1.

Succinimidyl 8-azido-3,6-dioxaoctanoate (17)

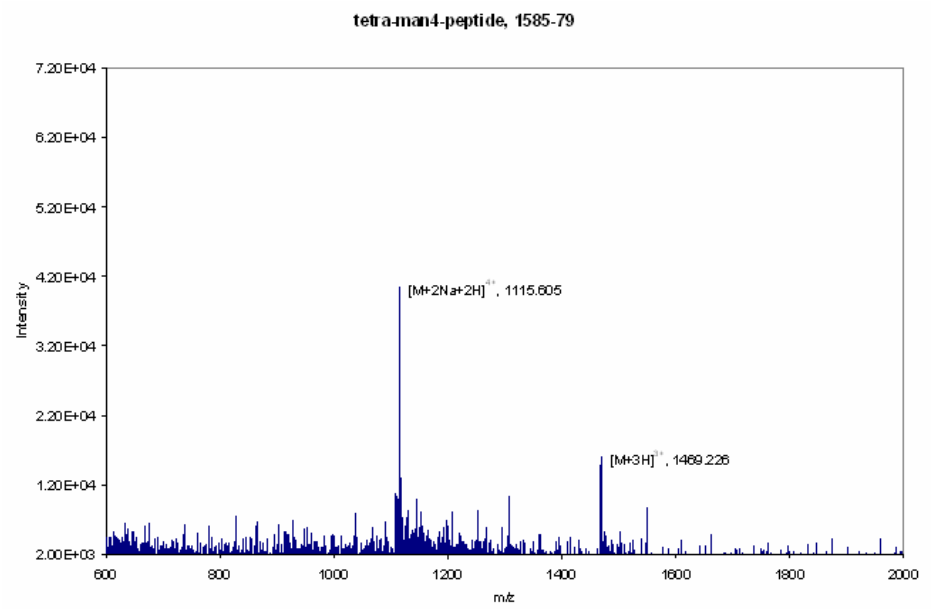
Compound **38** (370 mg, 1.51 mmol) was treated with TFA (2.5 mL) in CH_2Cl_2 (2.5 mL) for 2 h at rt. The mixture was concentrated to give the corresponding acid (260 mg, 90%) as a colorless oil. δ_H (500 MHz, $CDCl_3$): 9.50 (b, 1 H, COOH), 4.25 (s, 2 H, C*H*$_2$COOt-Bu), 3.83-3.72 (m, 6 H, $CH_2OCH_2CH_2O$), 3.45 (t, 2 H, J = 5.0 Hz, CH_2N_3); δ_C ($CDCl_3$, 125 MHz): 174.7, 71.2, 70.5, 70.1, 68.4, 50.6.

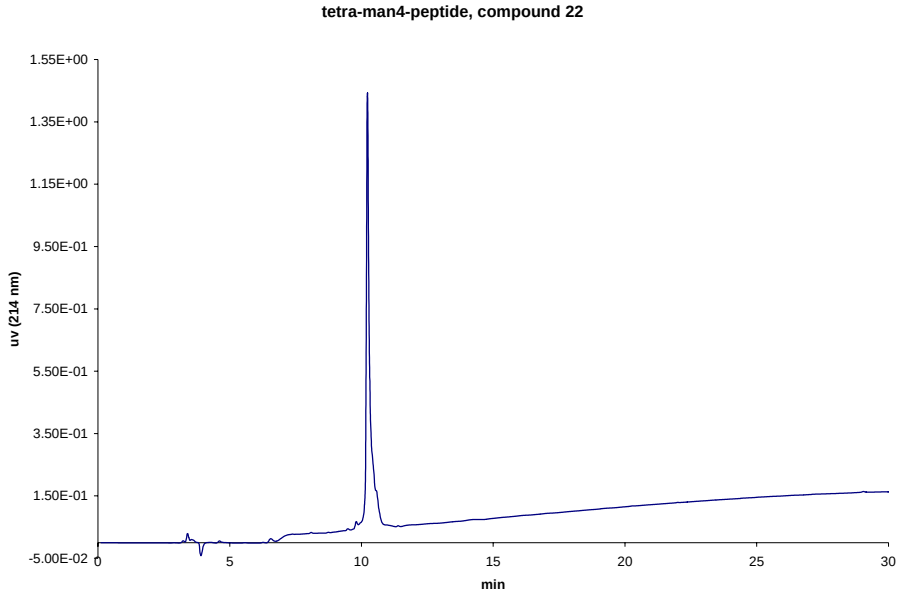
The above acid (100 mg, 0.529 mmol) and NHS (73 mg, 0.63 mmol) were dissolved in dry CH_2Cl_2 (10 mL) and the solution was cooled to 0 ºC, to which EDCI (120 mg, 0.63 mmol) was added. The mixture was stirred at rt for 4 h and concentrated to give crude **17** which was used in next step without further purification.





HPLC conditions: the analytical RP-HPLC was carried out on a C18 column (3.9x150 mm) at 40 ^{o}C. The column was eluted with a linear gradient of 0-90% MeCN containing 0.1% TFA at a flow rate of 1 mL/min over 30 min, and the glycopeptides were detected at UV 214 nm.





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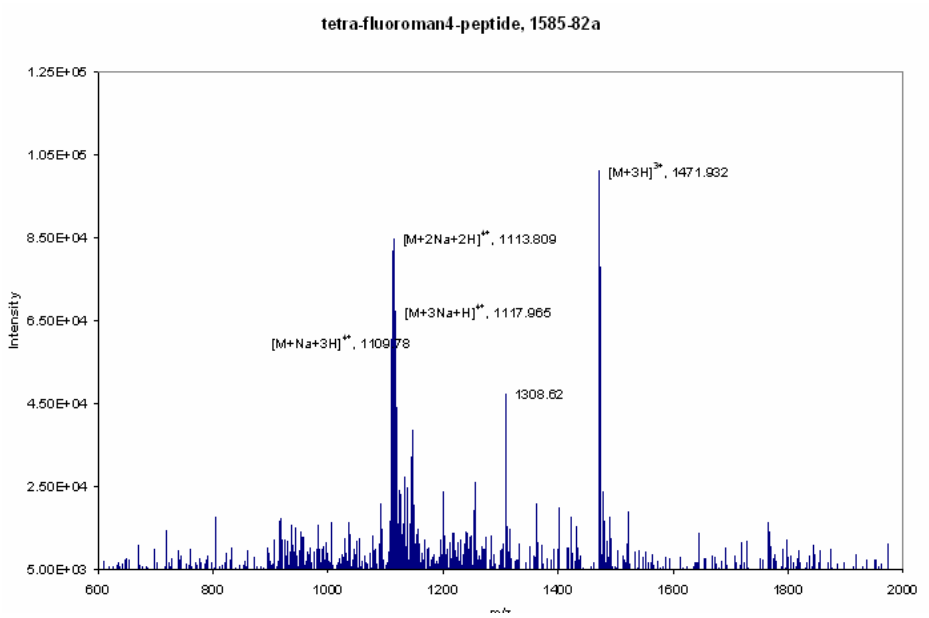

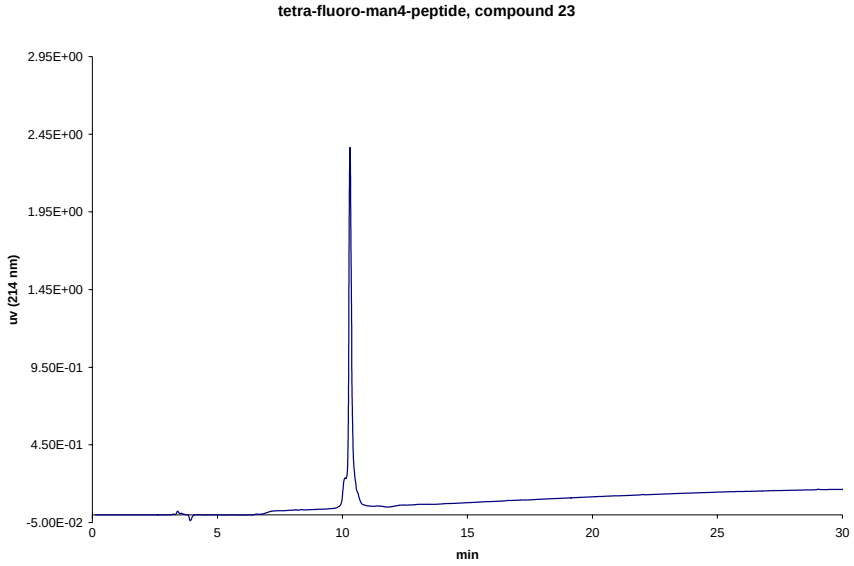

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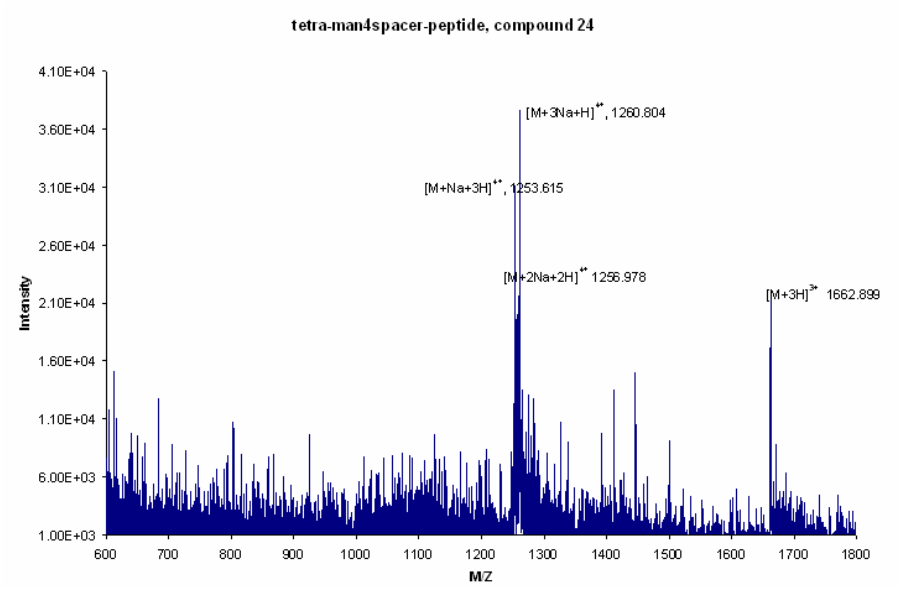

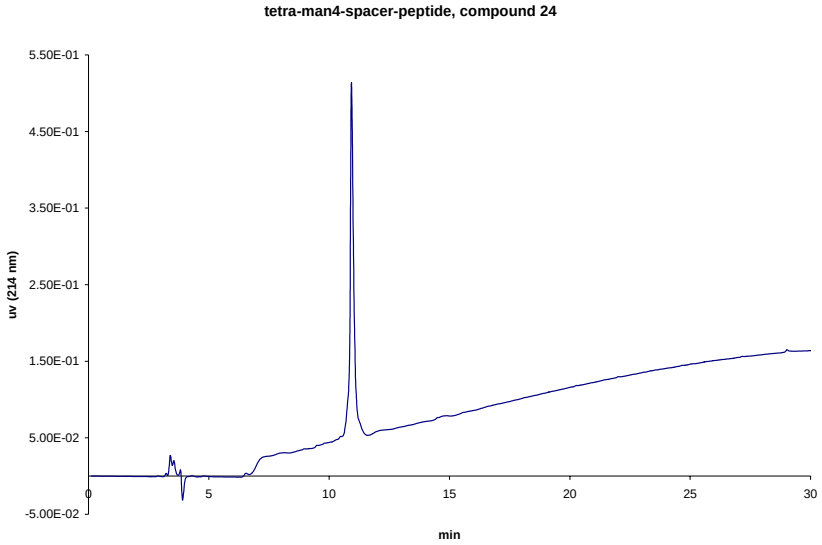

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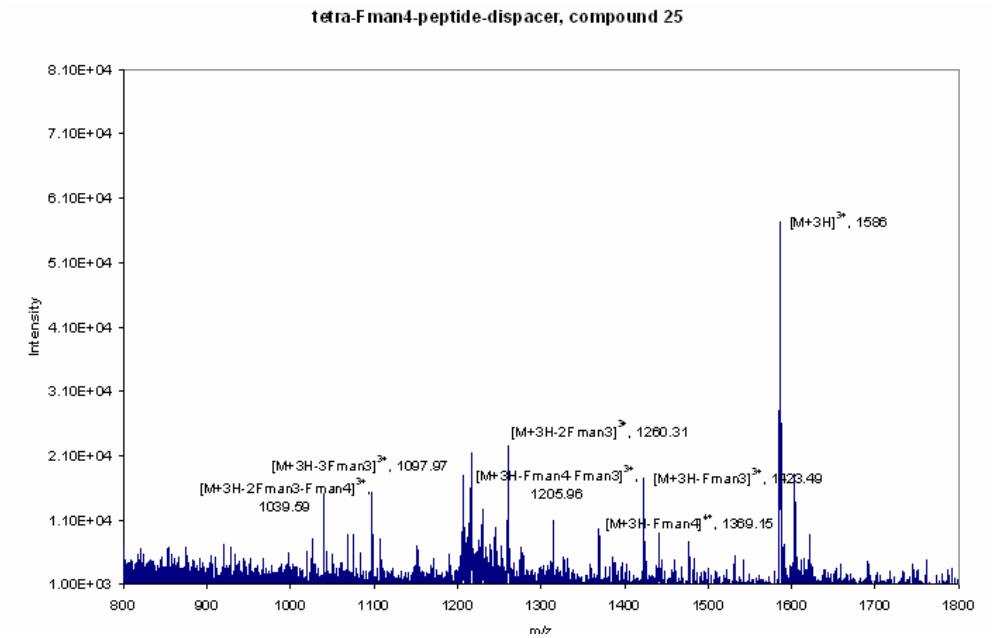
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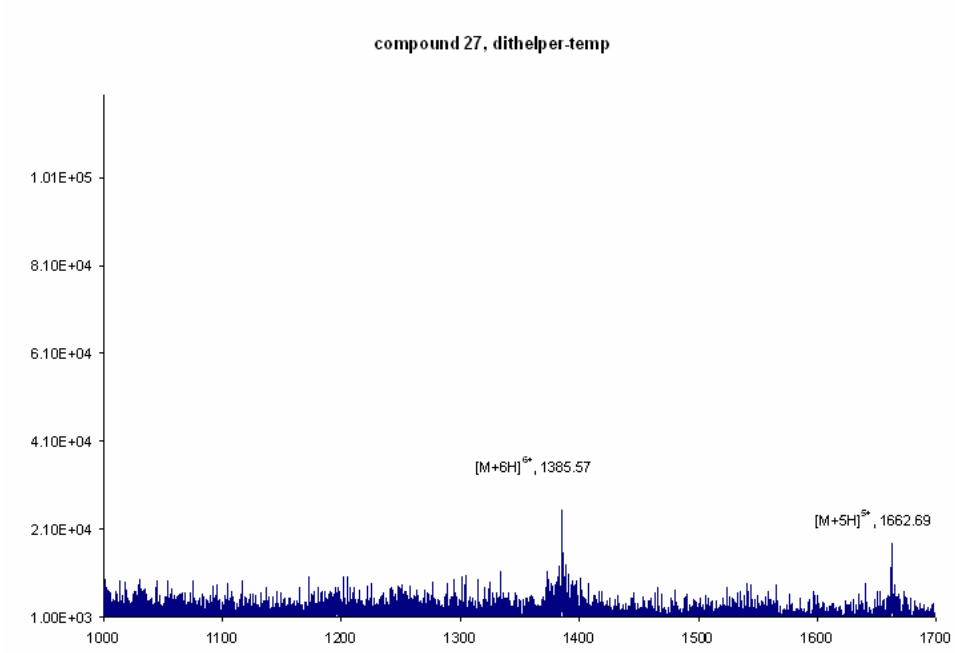

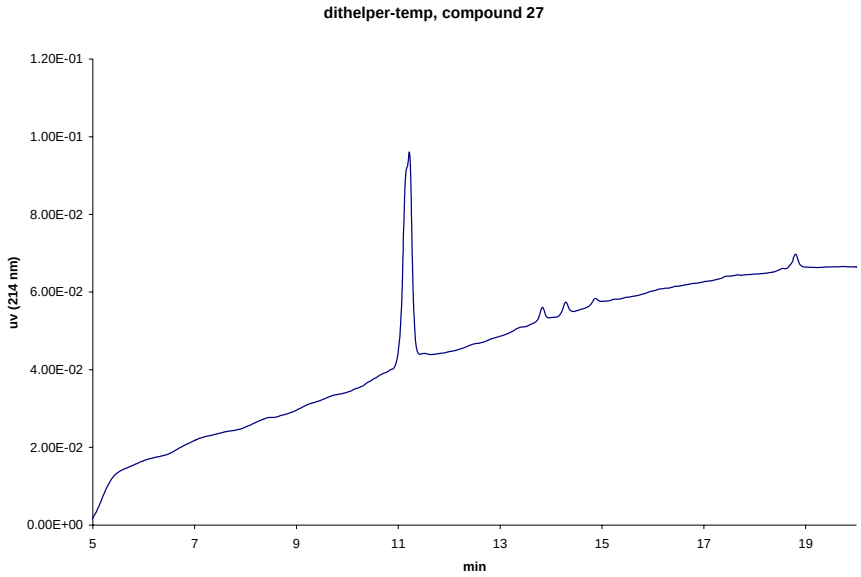

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